whether S100A8 and/or S100A9 could stimulate M2-macrophages, giving them a more pro-inflammatory character.
Methods: Monocytes were isolated from blood of healthy volunteers by isolating peripheral blood mononuclear cells (PBMCs) using a Ficoll-gradient followed by isolating CD14+ monocytes using magnetic-activated cell sorting (MACS^{TM}). CD14+ monocytes were differentiated into M1- or M2-macrophages by 6 day stimulation with either GM-CSF or M-CSF, respectively. mRNA levels of S100A8 and S100A9 in M1 and M2 macrophages were measured with RT-qPCR. Intracellular protein production was determined using FACS analysis and S100A8/A9 secretion was measured using an ELISA. M1 and M2 macrophages were stimulated with human recombinant S100A8 and S100A9. M1 and M2 markers (IL-1beta, TNFalpha, IL-6 and CD163, CD206 respectively) were measured using RT-qPCR, FACS and/or Luminex.
Results: S100A8 and S100A9 had a significantly higher mRNA expression (65- and 2.5-fold respectively, n=10) in human monocyte derived M1-macrophages compared to M2. Also, the levels of intracellular S100A8 and S100A9 were higher in M1 than in M2 macrophages (4.3-fold and 6.6-fold higher respectively, n=6). M1 macrophages secreted higher levels of S100A8/A9 heterodimer as compared to M2 macrophages (102.8 ng/ml vs 55.5 ng/ml respectively, n=10).
Stimulation of human monocyte derived M2-macrophages for 24 hours with S100A8 and S100A9 showed a marked upregulation of several pro-inflammatory markers: IL-1beta expression was increased by S100A8 and S100A9 on mRNA level (33- and 16-fold, n=10). At the protein level IL-6 and TNFalpha were strongly upregulated by S100A8 71- and 146-fold and by S100A9 93- and 121-fold, respectively (n=6).
Interestingly however, S100A8 or S100A9 stimulation did not alter the level of M2 membrane markers CD163 and CD206, both at mRNA as well as protein level.
Conclusions: Stimulation of M2 macrophages with S100A8 and S100A9, produced in high amounts by M1- macrophages, upregulates pro-inflammatory cytokines IL-1beta, IL-6 and TNF-alpha while M2 membrane markers CD163 and CD206 remain unchanged.
S100A8 and S100A9 production during OA could therefore contribute to synovial activation by stimulating the M2 macrophage towards a more pro-inflammatory phenotype.

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MYD88-DEPENDENT TLR-2/4 SIGNALING IN OSTEOARTHRITIS: PROTECTIVE OR NOT?

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Purpose: Toll-like receptor 2 (TLR-2)/TLR-4-mediated innate immunity modulates tissue remodeling and repair responses to endogenous ligands released during chronic, low-grade inflammation in osteoarthritis (OA). Furthermore, stimulation of macrophages with TLR ligands has been shown to induce superoxide dismutase-2 (SOD2), indicating a role of innate immunity in maintenance of redox homeostasis. Our lab has previously demonstrated impaired catabolic responses with MyD88 deficiency and TLR-2/4 combined deficiency in chondrocytes in vitro, although the direct roles of these proteins in in vivo OA pathogenesis remain unclear. Thus, we sought to determine if TLR-2/4 combined deficiency or MyD88 deficiency protects mice from OA induction via medial meniscectomy and whether chondrocytes deficient in MyD88 or TLR-2/4 demonstrate deficiencies in redox mediators.
Methods: MyD88 knockout (MyD88 KO) and TLR-2/TLR-4 double knockout (TLR-2/4 DKO) animals underwent survival medial meniscectomy surgery at two months of age, and were euthanized 8wks after. Their knee joints were isolated, parafinized, and sectioned, and OA was analyzed histologically via safranin-O staining relative to wildtype (WT) controls via the Chambers scoring system (MyD88: n = 5KO, 5WT. TLR-2/4: n = 9KO, 6WT; Mann-Whitney U Test with two tailed p-values). Expression of antioxidants SOD2, catalase and thioredoxin was assessed via western blot in mature chondrocytes isolated from mouse femoral head cartilage, and via immunohistochemistry on mouse knee sections from WT and MyD88 or TLR-2/4 deficient animals.
Results: Given the known decreases in catabolic responses in vitro with MyD88 or combined TLR-2/4 deficiency, we expected a significant protection from OA development in these mice relative to WT controls. However, both MyD88 KO and TLR-2/4 DKO animals demonstrated only a mild to moderate protection against OA (Table). More intriguing, anti-oxidant protein expression of SOD2, catalase and thioredoxin were all significantly decreased in unstimulated MyD88 KO and TLR-2/4 DKO chondrocytes and mouse knee sections, relative to WT controls.
Conclusions: To our knowledge, this is the first report of a loss of redox mediators in chondrocytes with innate immune deficiency, and suggests a previously unknown role of innate immunity in maintaining redox homeostasis in chondrocytes. A loss of redox homeostasis may explain the lack of a strong protective response against OA induction in mice deficient in MyD88 or in TLR-2 and TLR-4. Taken together, these data suggest an interesting dichotomy for the role of innate immunity in OA pathogenesis. Although TLR-2/4 and MyD88 signal endogenous ligand-induced catabolic responses and promote chronic low-level inflammation in cartilage chondrocytes, they also seem necessary for maintaining chondrocyte redox homeostasis. We believe this data offers new and significant insight into OA pathogenesis and signaling of chronic inflammatory responses in chondrocytes.

MyD88 and TLR-2/4 deficiency offer moderate protection against OA

Avg. Chambers Scores +/- SEM (OA knees)	KO	WT	p-values KO vs. WT
MyD88 Lateral Compartment	2.0 +/- 0.14	2.6 +/- 0.10	$p < 0.0001$
MyD88 Medial Compartment	6.5 +/- 0.27	8.1 +/- 0.26	$p < 0.0001$
TLR-2/4 Lateral Compartment	1.1 +/- 0.10	2.1 +/- 0.11	$p = 0.0952$
TLR-2/4 Medial Compartment	3.5 +/- 0.18	4.1 +/- 0.18	$p = 0.028$

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MEDIATORS OF THE INFLAMMATORY RESPONSE TO JOINT REPLACEMENT DEVICES

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Purpose: Several inflammatory, autoimmune and infective conditions cause the destruction of the weight bearing surfaces of major joints causing arthritis. Over one million people in the United States each year undergo a total joint arthroplasty, which is the prosthetic replacement of these, destroyed native bearing surfaces. A commonly utilized bearing surface material is ultra high molecular weight polyethylene (UHMWPE). Over time different size particles of wear debris are generated from the UHMWPE implant. These wear debris are responsible for the initiation of an aseptic inflammatory response known as osteolysis. The purpose of this study is the analysis of the innate immune mechanism utilized by macrophages, dendritic cells and osteoclasts in recognition of UHMWPE wear debris.
Methods: UHMWPE wear debris and polymers were characterized by transmission electron microscopy, mass spectrometry and Fourier transform infrared spectroscopy. Osteoclasts, macrophages and dendritic cells activation through the TLR1 and TLR2 signaling pathways was investigated using gene chip analysis, ko mice and in vitro UHMWPE binding to recombinant TLR2 molecules. The Nalp3 inflammasome assembly was investigated using Nalp3 ko mice, IL-1 and IL-18 ELISA assays
Results: We identified that the process of “in vivo” UHMWPE breakdown generates short alkane polymers in addition to the previously reported nanometers and micrometers size particles; the majority of these polymers presented side chain modifications consisting of aldehyde, ketonic and hydroxyl groups. Alkane side chains oxidation greatly enhanced their ability to activate dendritic cells as compared to the general inertness of the non-oxidized polymers. We also determined that a mixture of oxidized alkane polymers triggered a pro-inflammatory response upon binding to TLR2 homodimers and TLR1/2 heterodimers. This is the first report of a synthetic polymer engaging a TLR on immune cells. Additionally we identified the Nalp3 inflammasome assembly, following phagocytosis of

wear debris and endosomal destabilization, or plasma membrane frustrated phagocytosis, as an additional mechanism for UHMWPE recognition by the immune system.

Conclusions: Our data elucidate the mechanisms by which wear debris of different size, shape and composition can elicit an innate, immune response. Frustrated phagocytosis in response to micro particles, or endosomal destabilization as a result of phagocytosis of nanoparticles, are two of the major pathways through which wear debris activate the NALP3 inflammasome. Activation of Toll-like receptor (TLR) 2 and TLR4 are also pathways by which wear debris induce an inflammatory response. Ultimately, this multifaceted innate immune response will increase osteoclastogenesis and promote bone erosion.

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HOFFA-SYNOVITIS AND EFFUSION-SYNOVITIS ARE ASSOCIATED WITH KNEES UNDERGOING TOTAL KNEE REPLACEMENT: DATA FROM THE OSTEOARTHRITIS INITIATIVE

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Purpose: Hoffa-synovitis and effusion-synovitis, which may be assessed on non-contrast enhanced MRI, have been identified as important disease features related to pain and structural progression of knee osteoarthritis (OA). Change in these inflammatory imaging markers is positively associated with pain. As the indication for total knee joint replacement (TKR) is primarily based on clinical parameters, these two inflammatory imaging markers that correlate closely with the clinical disease manifestations are promising structural candidate markers for TKR. This study used a matched case-control design to determine if Hoffa- and effusion-synovitis are associated with TKR cross-sectionally and longitudinally.

Methods: Participants were drawn from the Osteoarthritis Initiative (OAI), a multicenter observational study, including 4796 participants with, or at risk of knee osteoarthritis. We studied knees from 121 OAI participants that underwent TKR before the 48 month visit for the time point prior to TKR, i.e. "T0" (e.g. for a TKR reported at the 48 month (M) visit, T0 = 36M); and 121 control knees that did not undergo TKR that were matched for radiographic disease stage, gender, and age within 5 years and were assessed at the same T0 follow-up visit. MR images were acquired at four OAI clinical centers using dedicated Siemens Trio 3 T scanners. The coronal intermediate weighted (IW) 2D turbo spin-echo (TSE), the sagittal 3D dual echo at steady state (DESS) sequence, coronal and axial multiplanar reformations of the 3D DESS and a sagittal IW fat suppressed TSE sequence were used for semiquantitative assessment. MRIs were read for Hoffa- and effusion-synovitis using the semiquantitative MOAKS system, which scores both features from 0-3 (0 being normal and 3 severe structural change - see Figures 1 and 2).

Conditional logistic regression was applied to assess the association with TKR at T0. In addition, any worsening (increase in at least one grade from baseline to follow-up) in Hoffa- and effusion-synovitis from the time point prior T0 (= T-1) to T0 was analyzed to assess the association with TKR following T0.

Results: Subjects were on average 65.3 years old (SD ± 8.6), predominantly female (58.1%) and overweight (mean BMI 29.6 SD ± 4.9).

In the cross-sectional comparison at T0 (the visit just prior to TKR), knees that underwent TKR were more likely to have any effusion-synovitis at T0 when compared to matched non-TKR knees (OR 2.45 95% confidence interval [CI] 1.22-4.95). No significant associations were found for presence of Hoffa-synovitis (Table 1). In the longitudinal analysis, knees that underwent TKR were more likely to have worsening of Hoffa- (OR= 7.0, [1.59,30.80]) and effusion-synovitis (OR= 2.27, [1.11,4.62]) from T-1 to T0 compared to matched non-TKR knees (Table 1).

Conclusions: Knees undergoing TKR are more likely to have effusion-synovitis compared to non-TKR knees. Worsening of both, Hoffa- and effusion-synovitis was also more likely among TKR knees. Presence and change of these imaging markers may be important prognostic markers with regard to the clinical outcome of knee OA using TKR as the outcome measure.

Table 1. Cross-sectional and longitudinal comparison of Hoffa- and effusion-synovitis in TKR knees vs. matched non-TKR knees

MRI biomarker	N (%)	Odds of MRI biomarker abnormality for TKR compared to non-TKR knees Crude Odds Ratio (95% confidence intervals)
Hoffa-synovitis at T0		
0	42 (17.5)	Reference
1	124 (51.7)	0.87 (0.44,1.73)
2	66 (27.5)	2.25 (0.97, 5.19)
3	8 (3.3)	2.15 (0.44,10.52)
Effusion-synovitis at T0		
0	50 (20.7)	Reference
1	65 (26.9)	1.05 (0.45,2.46)
2	79 (32.6)	2.78 (1.21,6.38)
3	48 (19.8)	8.21 (2.90,23.21)
Worsening of Hoffa- synovitis from T-1 to T0		
No change or improvement	193 (91.9)	Reference
Worsening by at least one grade	17 (8.1)	7.0 (1.59,30.80)
Worsening of effusion synovitis from T-1 to T0		
No change or improvement	169 (80.5)	Reference
Worsening by at least one grade	41 (19.5)	2.27 (1.12,4.62)

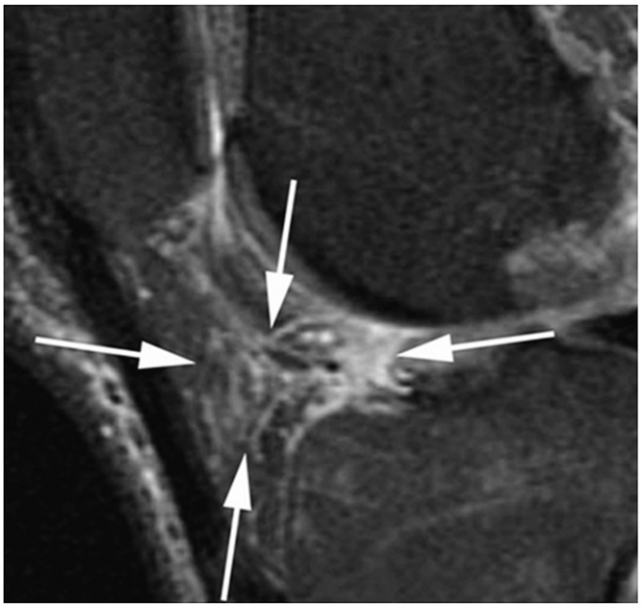

Figure 1.

Example of Hoffa-synovitis (grade 2). Marked hyperintensity in Hoffa's fat pad is depicted on sagittal IW fat suppressed image.